

## CLAIMS

**What is claimed is:**

1. An isolated HY2 family bilin reductase comprising an amino acid consensus sequence as illustrated in Figure 5 or in Figure 10 and having bilin reductase activity.

10 2. The bilin reductase of claim 1, wherein said bilin reductase is not hvrccr or atrccrl.

3. The bilin reductase of claim 1, wherein said bilin reductase is not rccr\_horvu or rccr\_arath.

15 4. The bilin reductase of claim 1, wherein said bilin reductase is not ycp2\_sympy or ycp3\_sympy.

5. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 50% sequence conservation as shown in Figure 10.

6. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 70% sequence conservation as shown in Figure 10.

20 7. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 90% sequence conservation as shown in Figure 10.

8. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 80% sequence conservation as shown in Figure 5.

25 9. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 100% sequence conservation as shown in Figure 5.

10. The bilin reductase of claim 1, wherein said bilin reductase is PebA.

11. The bilin reductase of claim 1, wherein said bilin reductase is PebB.

12. A ferredoxin-dependent bilin reductase comprising at least 15% sequence identity with an enzyme selected from the group consisting of HY2\_ARATH,

5 YCP2\_SYNPY, YHP2\_PROMA, YHP3\_PROMA, YCP3\_SYNPY, SLR0116,  
PcyA\_ANASP, PcyA\_NOSPU, PcyA\_SyNY3, PcyA\_SYN8.1, PcyA\_PROME,  
PebA\_SYNPY, PebA\_SYN8.1, PebA\_PROMA, PebA\_PROME, PewbB\_NOSPU,  
HY2\_ARATH, RCCR\_ARATH, and RCCR\_HORVU, and where, when aligned with HY2,  
comprises conserved hydrophobic residues at position 137, 157, 158, 256, and 314.

10 13. The bilin reductase of claim 12, wherein said bilin reductase, when  
aligned with HY2, comprises a residue selected from the group consisting of Pro-151, Phe-  
221, Ser222, and ASP-171.

14. The bilin reductase of claim 13, wherein said bilin reductase, when  
aligned with HY2, comprises Pro-151, Phe-221, Ser-222, and ASP-171.

15 15. The bilin reductase of claim 12, wherein said bilin reductase is not  
hvrcr or atrccr1.

16. The bilin reductase of claim 12, wherein said bilin reductase is not  
rccr\_horvu or rccr\_arath.

20 17. The bilin reductase of claim 12, wherein said bilin reductase is not  
ycp2\_synpy or ycp3\_synpy.

18. The bilin reductase of claim 12, wherein said bilin reductase is not  
HY2.

19. An isolated bilin reductase having bilin reductase activity and  
comprising an amino acid sequence of polypeptide selected from the group consisting of  
25 HY2, athy2, slr0116, c362\_anab, ycp2-synpy, ycp3\_ssynpy, PcyA\_ANASP, PcyA\_NOSPU,  
PcyA\_SYNY3, PcyA\_SYN81, PcyA\_PROME, PebA\_SYNPY, PebA\_SYN81,  
PebA\_PROMA, PebA\_PROME, PebA\_NOSPU, PebB\_SYNPY, PebB\_SYN81,  
PebB\_PROMA, PebB\_PROME, PebB\_NOSPU, HY2\_ARATH, RCCR\_ARATH, and  
RCCR\_HORVU, or conservative substitutions thereof.

30 20. The bilin reductase of claim 19, wherein said bilin reductase  
comprises an amino acid sequence of a polypeptide selected from the group consisting of  
athy2, slr0116, c362\_anab, ycp2-synpy, ycp3\_ssynpy, PcyA\_ANASP, PcyA\_NOSPU,

5 PcyA\_SYNY3, PcyA\_SYN81, PcyA\_PROME, PebA\_SYNPY, PebA\_SYN81,  
PebA\_PROMA, PebA\_PROME, PebA\_NOSPU, PebB\_SYNPY, PebB\_SYN81,  
PebB\_PROMA, PebB\_PROME, PebB\_NOSPU, HY2\_ARATH, RCCR\_ARATH, and  
RCCR\_HORVU.

10 21. A method of converting a biliverdin to a phytobilin, said method  
comprising contacting a bilin reductase of claim 1, with a biliverdin whereby said biliverdin  
is converted to a phytobilin.

22. The method of claim 19, wherein said bilin reductase is a  
cyanobacterial bilin reductase.

15 23. The method of claim 19, wherein said bilin reductase is an algal bilin  
reductase.

24. The method of claim 19, wherein said bilin reductase is a plant bilin  
reductase.

25. The method of claim 21, wherein said bilin reductase is  
recombinantly expressed.

20 26. The method of claim 21, wherein said contacting is *ex vivo*.

27. The method of claim 21, wherein said contacting is in a cell and said  
bilin reductase is a heterologous polypeptide.

28. The method of claim 21, further comprising contacting said  
phytochromobilin with a second bilin reductase to produce a phytochrome.

25 29. The method of claim 21, further comprising contacting said  
phytochromobilin with a second bilin reductase to produce a phytofluor.

30. The method of claim 29, wherein said second bilin reductase is PebB.

31. The method of claim 21, wherein said bilin reductase is ycp2-snpy.

32. The method of claim 29, wherein said bilin reductase is ycp3-snpy.

5           33. A nucleic acid comprising a nucleic acid encoding a bilin reductase of any one of claims 1 through 20.

10          34. The nucleic acid of claim 33, wherein said nucleic acid is a vector.

15          35. A cell comprising a heterologous nucleic acid comprising a nucleic acid encoding a bilin reductase of any one of claims 1 through 20.

20          36. The cell of claim 35, wherein said cell is selected from the group consisting of an algal cell, a plant cell, a yeast cell, a bacterial cell, an insect cell, and a mammalian cell.

25          37. A nucleic acid comprising a nucleic acid that specifically hybridizes with a nucleic acid of any one of claims 1 through 20 under stringent conditions and that encodes a polypeptide having bilin reductase activity, wherein said nucleic acid does not encode an hvrccr or an atrccr polypeptide.

30          38. The nucleic acid of claim 37, wherein said nucleic acid is a vector.

35          39. A method of detecting expression of a polypeptide, said method comprising:

40              providing a cell comprising a nucleic acid encoding an apophytochrome; and a nucleic acid encoding a bilin reductase that produces a phytobilin that assembles with said apophytochrome to produce a phytofluor;

45              and detecting an optical signal produced by said phytofluor.

50          40. A method of producing a photoactive holophytochrome, said method comprising:

55              co-expressing in a cell:

60                  a heme oxygenase;

65                  an apophytochrome;

70                  and a ferredoxin-dependent bilin reductase;

75          30 whereby said cell produces said photoactive holophytochrome and where one or more of said apophytochrome and said ferredoxin-dependent bilin reductase are expressed by heterologous nucleic acids.

5           41.     The method of claim 40, wherein said cell is selected from the group consisting of an algal cell, a yeast cell, a bacterial cell, a plant cell, an insect cell, and a mammalian cell.

42.     The method of claim 40, wherein said ferredoxin-dependent bilin reductase is an HY2 family bilin reductase.

10           43.     The method of claim 40, wherein said apophytochrome and said ferredoxin-dependent bilin reductase are both expressed by heterologous nucleic acids.

44.     The method of claim 40, wherein said heme oxygenase is expressed by a heterologous nucleic acid.

15           45.     The method of claim 40, wherein said photoactive holophytochrome is not a phytofluor.

46.     The method of claim 45, wherein said ferredoxin-dependent bilin reductase is an HY2 family member.

47.     The method of claim 45, wherein said ferredoxin-dependent bilin reductase is *HY2*.

20           48.     The method of claim 45, wherein said ferredoxin-dependent bilin reductase is *pcyA*.

49.     The method of claim 40, wherein said photoactive holophytochrome is a phytofluor.

25           50.     The method of claim 49, wherein said apophytochrome is expressed as a fusion protein with a protein that is to be labeled with said phytofluor.

51.     The method of claim 49, wherein said method comprises expressing the ferredoxin-dependent bilin reductase *pebA* or *pebB*.

52.     The method of claim 51, wherein said method comprises expressing both ferredoxin-dependent bilin reductase *pebA* and *pebB*.

30           53.     The method of claim 51, wherein said cell is a bacterial cell.

5           54.     The method of claim 40, wherein said method further comprises recovering said photoactive holophytochrome from said cell.

10           55.     A cell comprising:  
                  a heme oxygenase;  
                  an apophytochrome;  
                  and a ferredoxin-dependent bilin reductase;

whereby said cell produces a photoactive holophytochrome and where one or more of said apophytochrome and said ferredoxin-dependent bilin reductase are expressed by heterologous nucleic acids.

15           56.     The cell of claim 55, wherein said cell is selected from the group consisting of an algal cell, a yeast cell, a bacterial cell, a plant cell, an insect cell, and a mammalian cell.

57.     The cell of claim 55, wherein said ferredoxin-dependent bilin reductase is an HY2 family bilin reductase.

20           58.     The cell of claim 55, wherein said apophytochrome and said ferredoxin-dependent bilin reductase are both expressed by heterologous nucleic acids.

59.     The cell of claim 55, wherein said heme oxygenase is an endogenous heme oxygenase.

60.     The cell of claim 55, wherein said heme oxygenase is expressed by a heterologous nucleic acid.

25           61.     The cell of claim 55, wherein said photoactive holophytochrome is not a phytofluor.

62.     The cell of claim 61, wherein said ferredoxin-dependent bilin reductase is an HY2 family member.

30           63.     The cell of claim 61, wherein said ferredoxin-dependent bilin reductase is *HY2*.

5           64. The cell of claim 61, wherein said ferredoxin-dependent bilin  
reductase is *pcyA*.

10          65. The cell of claim 55, wherein said photoactive holophytocrome is a  
phytofluor.

15          66. The cell of claim 65, wherein said apophytocrome is expressed as a  
fusion protein with a protein that is to be labeled with said phytofluor.

20          67. The cell of claim 65, wherein said cell comprises expressing the  
ferredoxin-dependent bilin reductase *pebA* or *pebB*.

25          68. The cell of claim 67, wherein said cell comprises expressing both  
ferredoxin-dependent bilin reductase *pebA* and *pebB*.

30          69. The cell of claim 67, wherein said cell is a bacterial cell.

20          70. A recombinant nucleic acid comprising:  
                  a nucleic acid encoding a heme oxidoreductase; and  
                  a nucleic acid encoding and a ferredoxin-dependent bilin reductase;  
where said nucleic acid expresses a functional heme oxidoreducase and a functional bilin  
reductase.

25          71. The nucleic acid of claim 70, wherein said heme oxidoreductase and  
said bilin reductase are under control of the same promoter.

30          72. The nucleic acid of claim 71, wherein said promoter is a constitutive  
promoter.

25          73. The nucleic acid of claim 71, wherein said promoter is an inducible  
promoter.

30          74. The nucleic acid of claim 71, wherein said promoter is a tissue-  
specific promoter.

30          75. The nucleic acid of claim 70, wherein said nucleic acid is present in a  
cell.

5           76.     The nucleic acid of claim 75, wherein said cell is selected from the group consisting of an algal cell, a bacterial cell, a plant cell, a yeast cell, a mammalian cell, and an insect cell.

77.     The nucleic acid of claim 75, wherein said nucleic acid comprises a gene selected from the group consisting of *HO1*, *HY2*, *PcyA*, *PebA*, and *PebB*.

10        78.     The nucleic acid of claim 77, wherein said nucleic acid comprises an *HO1* coding region and a *pcyA* coding region.

79.     The nucleic acid of claim 78, wherein said nucleic acid further comprises a *pcyB*.